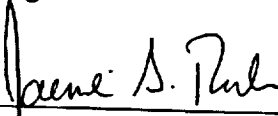


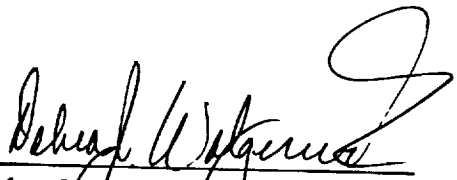
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Final Technical Report
NASA NAG2-1185
Debra J. Wolgemuth, Ph.D., P.I.

I. Details of Award

- A. Performance site: Health Sciences, Columbia University in the City of New York
- B. Title: Effects of Space Flight-Associated Stimuli on Development of Murine and Medaka Sensory-Motor Systems
- C. Principal Investigator: Debra J. Wolgemuth, Ph.D.
- D. Dates covered: 1/1/98-6/30/99 (no cost extension to 9/30/99)
- E. Report submitted to: NASA AMES Space Center
- F. Date submitted: February 16, 2000
- G. Statement of inventions: No inventions or patents resulted from the work under this grant.
- H. Signatures:


Jaime S. Rubin, Ph.D.
Office of Grants and Contracts


Debra J. Wolgemuth, Ph.D.
Principal Investigator

II. Overview

The major goal of these studies was to continue investigations into the influence of altered gravitational fields on the development and function of the vertebrate brain and nervous system. Of major focus during the 18-month funding period of this award was the maintenance of the animals used in the experimental mouse and medaka model paradigms. The experiments focused on characterization of stress-sensitive periods in neural development and immediate or delayed effects on gene expression, physiology and behavior. The hypothesis under investigation was that the environment of space will have biologically significant effects on the development and function of the vertebrate nervous system. We have postulated that these effects will be more significant on certain neural compartments, such as the vestibular-motor system, and that these effects will have greater impact at particular stages of embryonic and post-natal development of the animal. Development of the central nervous system is well known for its vulnerability and sensitivity to environmental stimuli, although the effects of gravitational influences are poorly understood. The long-term goals of this research effort, initiated previously and continued in limited capacity during this interim period, were to provide important new information on the effects of altered environments during these critical periods.

III. Specific Goals of the Project

1. Maintain inbred strains of mice and fish that serve as the source of experimental models.
2. Continue to analyze by in situ hybridization and immunohistochemistry specimens that have been exposed to hypoxia or heat shock.
3. Continue analysis of gene expression in the developing medaka central nervous systems of homeobox genes, genes that may serve as sensitive molecular markers of altered environments.

IV. Summary of Research Findings

1. We have been able to continue to house the mice and medaka colonies, albeit at a maintenance level.
2. We have made considerable progress in the analysis of the expression of the small heat shock protein, Hsp25, during neural development and importantly, in response to two environmental stresses, hypoxia and heat shock. Although expression of the small heat shock protein family member Hsp25 had been previously observed in the central nervous system (CNS), both constitutively and upon induction, its function in the CNS remains far from clear. We have characterized the spatial pattern of expression of Hsp25 in the normal adult mouse brain as well as the changes in expression patterns induced by subjecting mice to experimental hyperthermia or hypoxia. Immunohistochemical analysis revealed a surprisingly restricted pattern of constitutive expression of Hsp25 in the brain, limited to the facial, trigeminal, ambiguus, hypoglossal and vagal motor nuclei of the brain stem. After hyperthermia or hypoxia treatment, significant increases in the levels of Hsp25 were observed in these same areas and also in fibers of the facial and trigeminal nerve tracts. Immunoblot analysis of protein lysates from brain stem also showed the same pattern of induction of Hsp25. Surprisingly, no other area in the brain showed expression of Hsp25, in either control or stressed animals. The highly restricted expression of Hsp25 implies that this protein may have a specific physiological role in the orofacial motor nuclei, which govern precise coordination between muscles of mastication and the pharynx, larynx, and face. Its rapid induction after stress further suggests that Hsp25 may serve as a specific molecular chaperone in the lower cholinergic motor neurons and along their fibers under conditions of stress or injury.
3. We continued to analyze the expression of a medaka group 4 homeobox gene during medaka development. Briefly, homeobox-containing genes of the Hox class have been identified and characterized in several species. Their expression patterns and functions in vertebrates have been investigated extensively, especially in mouse, chicken and human. We have identified a Hox gene in the Japanese fish medaka and its expression pattern as well as its response to retinoic acid has been investigated. Sequence analysis showed that this medaka gene had the highest homology to the

group 4 class of vertebrate Hox genes. Northern blot and in situ hybridization analysis revealed that the gene in medaka is expressed with a distinct spatial and temporal pattern during embryogenesis, similar to its potential mammalian counterpart. Its anterior boundary was located in a position posterior to the otic vesicle in the central nervous system, very similar to what has been observed in mouse. Since retinoic acid is widely regarded as a Hox gene regulator and retinoic acid response elements were found in the upstream region of this medaka group 4 Hox gene, medaka embryos were treated with retinoic acid at different stages of development. The anterior boundary of expression in the central nervous system of this group 4 Hox gene was shifted anteriorly after retinoic acid treatment, analogous to the phenomenon observed in in vivo experiments of two of the group 4 murine Hox genes. Our experiments show that the medaka group 4 Hox gene possesses many same characteristics as those of Hox genes in mouse and human, underscoring the remarkable conservation of expression and possible function of these genes.

4. Our studies on Hsp25 were extended to include its interaction with the stress- and mitogen-activated protein (MAP) kinase, p38, which can be activated by a broad array of environmental stresses as well as by inflammatory cytokines and tumor necrosis factor. The p38 kinase pathway may lead to phosphorylation and activation of different factors necessary for the stress response, including ATF1, ATF2, CHOP, CREB, MAP kinase activated protein kinase-2/3 (MAPKAPK-2/3) and Hsp25. Phosphorylation of Hsp25 by p38 kinase has been implicated in the signal transduction pathway by which non-neuronal cells regulate organization of their actin filament system, suggesting that Hsp25 can act as an actin-capping protein. Furthermore, during stress, Hsp25 may interact with Akt/PKB kinase, a member of PI-3 kinase pathway that prevents neuronal cell death. The experimental system used spinal cords of axotomized, sham-operated and control mice which were dissected and examined using immunohistochemistry. Protein lysates were analyzed by Western blot analysis and immunoprecipitation. Expression of p38 kinase was found to be induced in the lumbar spinal cord on the side of the injury on the third day postaxotomy, to reach maximum levels on the fourth day, and to be sustained up to 20 days postaxotomy. Antibodies specific for phosphorylated p38 protein revealed that localization of phosphorylated p38 kinase was restricted to axotomized motor neurons. Interestingly, the expression patterns of MAPKAPK-2 and Akt kinases as well as Hsp25 protein was similar to that of p38. The p38 kinase inhibitor SB203580 downregulated expression of Hsp25. Immunoprecipitation showed that p38, Hsp25 and Akt formed protein complexes during regeneration. The results indicate that p38/Hsp25/Akt signaling pathway may be a critical part of regenerative mechanisms in the adult CNS. Together, our observations implicate Hsp25 as a central player in a complex system of signaling that might both promote regeneration of nerve fibers and prevent neuronal cell death in the injured spinal cord.
5. Hsp25 expression in the testes had not previously been examined within an extended developmental framework or with regard to testicular tissue type. We therefore characterized the developmental expression of Hsp25 in the testes and examined its spatial and temporal correlation of expression with p38 and p38 phosphorylation. We

found that Hsp25 expression appeared to be limited to the cytoplasm of Sertoli cells, and that p38 activation by phosphorylation did not appear to correspond to this pattern of expression. We observed Hsp25 expression in the testes at the first time point at which we were able to observe signs of differentiation in the seminiferous tubules - day 13.5 of embryonic development. Hsp25 did not appear either in the primordial germ cells or in cells surrounding the developing tubule, and was limited to the cytoplasm of maturing Sertoli cells. This pattern of expression continued for all subsequent time points examined. Expression in the adult appeared to be evenly distributed along the Sertoli cells rather than concentrated about the actin-rich basal cytoplasm, as might be expected from Hsp25's putative role as a modulator of actin dynamics. There was a large apparent increase in staining intensity for Hsp25 lasting from E18.5 to post-natal day 3 corresponding to the period of testicular cord organization prior to the rapid proliferation of Sertoli cells. Although it might have been expected that this upstream activator of Hsp25 would be found in the same portions of the testes as the heat shock protein, we did not find this to be the case for early embryonic development. While Hsp25 was expressed in Sertoli cells, p38 expression was limited to primordial germ cells until the 16th day of development, when it was first observed to appear in Leydig and Sertoli cells. This corresponds to the onset of rapid Sertoli cell proliferation. In sections of adult testes p38 was expressed solely in spermatogonia and not at all in spermatocytes or developing or elongating spermatids.

P38 is believed to be activated by phosphorylation at either or both of two tyrosine residues. We then employed an antibody for p38 detection that recognizes the biphosphorylated form only. We expected phospho-p38 to be a subset of our observed P38 expression, since the anti-p38 antibody was not phosphorylation specific. This was the case for all embryonic and juvenile tissue. As was observed for p38 expression, phospho-p38 was detected only in primordial germ cells during early embryonic development (until E15.5). Unlike our observations for p38, Sertoli cells did not show the presence of phospho-p38 after E15.5. Phospho-p38 is restricted to the primordial germ cells and Leydig cells for all time points examined until adulthood. The adult testes show an unusual staining pattern, which is not uniform across the testes or individual tubules, and which does not correspond to the staining observed for the non phospho-specific p38 antibody. It is possible that the epitope recognized by the phospho-p38 antibody is exposed only in p38's activated state, so that the non phospho-specific p38 antibody fails to detect it. The absence of p38 staining in certain regions where phospho-p38 stained positively might similarly be due to the epitope recognized by the p38 antibody being protected when the protein is in its phosphorylated form.

V. **Publications** (1/1/98 to 9/30/99)

Murashov, A.K., Talebian, S., and Wolgemuth D.J. Role of Heat Shock Protein Hsp25 in the Response of Orofacial Nuclei Motor System to Physiological Stress. (1998). **Mol. Brain Research** 63, 14-24.

Murashov, A.K., Haq, I., Park, E. and Wolgemuth D.J. Role of the Hsp25 and p38 kinase pathway in axonal regeneration of spinal motor neurons. In: 28th Annual Meeting Society for Neuroscience, Los Angeles, CA., November 7-12, 1998, p. 1845.

Murashov, A.K. and Wolgemuth D.J. Developmentally Regulated and Spatio-specific Expression of Hsp70.2 Antisense and Sense Transcripts in the Mouse Brain. In preparation.

Murashov, A.K., Bauer, R., Talebian, S., and Wolgemuth, D.J. Expression of heat shock proteins in mouse gut during heat shock and hypoxia. In preparation.

Murashov, A.K., Haq, I., Park, E. and Wolgemuth, D.J. Role of mitogen-activated signal transduction pathway and hsp25 in regeneration of spinal motor neurons. In preparation.

Wang, X., Herrada, G., Crotty D.A., and Wolgemuth D.J. Identification and Characterization of Expression of the Fish Medaka Homologue of a Group 4 Hox Gene. In preparation.